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The Alaskan Sled Dog

A Genetic Breed Apart

BY RAYMOND MACDOUGALL, NHGRI

Heather Huson has a passion for sled dogs. She grew up participating in sled-dog racing, qualifying twice for the U.S. Olympics team and competing in the sport's world championship of sled-dog racing in 1995 and 2001.

Now the National Human Genome Research Institute (NHGRI) graduate student and University of Alaska at Fairbanks Ph.D. candidate has found another place for sled dogs: in her genetics research.

Huson is the lead author of a study on the genetic origins of sled dogs, a study whose authors also include Elaine Ostrander and other scientists from NHGRI's Cancer Genetics Branch as well as her university advisor. In their analysis of 199 Alaskan sled dogs and 681 purebred dogs belonging to 141 other breeds, the study found that Alaskan sled dogs represent a distinct genetic breed characterized by performance and behavior rather than appearance. The study was published in the online issue of the BioMed Central's open-access journal *BMC Genetics* (*BMC Genet* **11**:71, July 22, 2010).

"The Alaskan sled dog presents a case in which a genetically distinct breed of dog has been developed through the selection and breeding of individuals based solely on their athletic prowess," Huson said. "Interestingly, this continual out-crossing for athletic enhancement has still led to the Alaskan sled dog repeatedly producing its own unique genetic signature. Indeed, the

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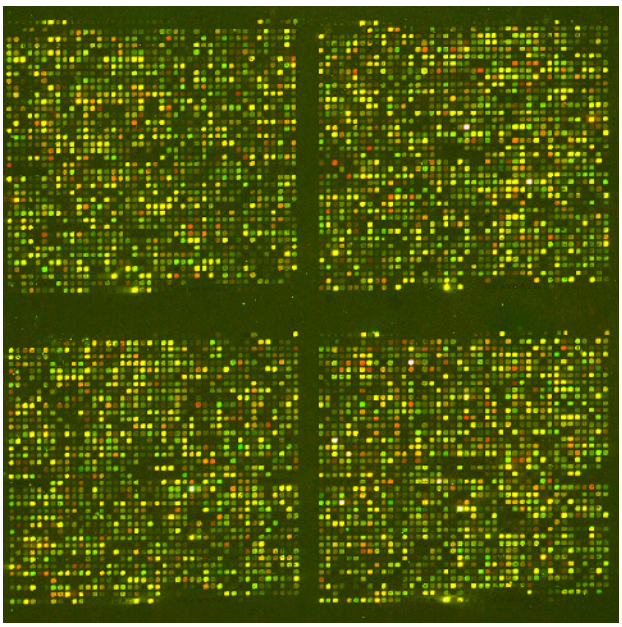
NIEHS Advances Predictive Genomics

And May Help Prevent Drug-Induced Liver Injury in the Process

BY THADDEUS SCHUG, NIEHS

Toxicogenomics pioneers at the National Institute of Environmental Health Sciences (NIEHS) are harnessing the power of microarrays and other gene-expression technologies to gain new insights into the mechanisms of toxicity and advance the discipline of predictive toxicology.



JUANITA MARTINEZ, ANGELINA RODRIGUEZ, U. OF NEW MEXICO

NIEHS scientists used microarrays to identify genetic indicators that could predict drug-induced liver injury. Each microarray is a small membrane or glass slide containing samples of many genes arranged in a regular pattern.

Take drug-induced liver injury (DILI) for instance. Although new drugs are rigorously tested as they are being developed, rare adverse effects—such as acute liver failure—might not become evident until after the drugs are approved. In fact, DILI is a major reason that drugs are withdrawn from the market, have their use restricted, or are required to wear a warning label. It is difficult to predict DILI or diagnose it in its early stages. Serum markers or liver biopsies are not true predictors and only detect liver injury once it's happened. And some people may be genetically predisposed to having an adverse reaction to certain drugs.

But NIEHS researchers may have found a better way to predict DILI, according to a study published in a special issue of *The Pharmacogenomics Journal*. Bioinformatician Pierre Bushel led a team of 23 investigators who used gene expression microarray technology to

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demonstrate that genomic indicators in the blood can predict drug-related liver injury long before it occurs (*The Pharmacogenomics J* **10**:267–277, 2010).

"There's a huge need to put better information in the hands of clinicians and genomics is an important tool for helping us reach that goal," said study coauthor Richard Paules, who is a principal investigator in NIEHS's Laboratory of Toxicology and Pharmacology and director of the Microarray Core facility.

GENOMIC INDICATORS PREDICT A PHENOTYPE OF TOXICITY

To test the utility of genomic indicators for predicting very early stage DILI, the team used a MicroArray Quality Control (MAQC)-II project dataset that was generated by the NIEHS National Center for Toxicogenomics (NCT) and contributed by Paules. It consisted of gene-expression data from blood and liver tissue in rats. The researchers analyzed genomic indicators in the blood and found they could predict liver necrosis or hepatic cell death across a variety of chemical compounds that target the liver. They confirmed their predictions by examining liver samples in rats after they developed DILI.

The investigators compared results on two microarray platforms for profiling the liver data and predicted DILI with 92 percent accuracy. In a validation component of the study, they also showed that these genomic biomarkers were highly accurate in being able to predict liver injury caused by acetaminophen as well as by two nontherapeutic chemical compounds.

STEVE MCCAW

STEVE MCCAW

Pierre Bushel (left), head of the NIEHS's Microarray and Genome Informatics Group, used microarray technology to demonstrate that genomic indicators in the blood are good predictors of drug-induced liver injury. This spring the Society of Toxicology recognized Richard Paules (right) with the 2010 Leading Edge in Basic Science Award "for his work in the integration of genomics into the investigation of the molecular basis of injury and disease processes," describing him as "a visionary . . . who has diligently positioned NIEHS at the forefront of the field."

"Our results strongly support the claim that genomic indicators in the blood can serve as biomarkers of necrosis," said Bushel. The findings suggest that they could be used "for diagnostic testing of DILI in humans."

The findings underscore the potential for using microarray gene expression technology to individually tailor drug treatments.

NIEHS has a long history of pioneering work in toxicogenomics, a field combining toxicology with genomics research. "While the challenges of developing signatures to utilize as clinical biomarkers of specific adverse effects are great, the prospects have never been brighter," said Paules. "We really hope to provide better science for better treatment of disease."

The study's findings underscore the potential for using microarray gene-expression technology to individually tailor drug-treatments and speed up the development of high-throughput drug screening assays.

MOVING FORWARD—THE SEQC PROJECT

The NIEHS-led study provides a proof of principle for the FDA-led MicroArray Quality Control (MAQC) project, which has been assessing bioinformatics for genome technologies in biomedical research. MAQC-II is developing best practices for using microarray-based technologies to predict toxicological and clinical endpoints.

Until recently, the potential of microarray analysis for addressing previously intractable problems—and uncovering novel potential targets for therapies—was hampered by studies with dissimilar or altogether contradictory results obtained using different microarray platforms to analyze identical RNA samples. In establishing best-practice guidelines, the MAQC-II project, which involves more than 200 scientists working in 36 teams, aims to establish a foundation for the reliable use of microarrays in clinical, research and regulatory settings and will be important as treatments are tailored to patients' individual needs.

A Decade of Toxicogenomics at NIEHS

In 2000, NIEHS established the National Center for Toxicogenomics (NCT) to promote the evolution and coordinated use of gene-expression technologies and to apply them to the assessment of toxicologic effects in humans.

The primary goal was to provide a worldwide reference system of genome-wide gene-expression data and to develop the Chemical Effects in Biological Systems (CEBS), a database that houses data—from academic, industrial, and governmental laboratories—of interest to environmental health scientists (http://www.niehs.nih.gov/research/resources/databases/cebs). A secondary goal was to expand the understanding of the mechanisms by which stressor-induced injury occurs.

NIEHS was also part of a Toxicogenomics Research Consortium (TRC) that worked under a National Institutes of Health cooperative agreement. TRC was a grant-supported consortium divided into two components—an independent component comprising individual research projects within the framework of a program project grant and a dependent component in which members of the consortium collaborated in the development of studies to bring definition to toxicogenomics.

NIEHS phased out NCT in 2006, but NIEHS researchers Richard Paules and Pierre Bushel continued their efforts to develop practical applications for toxicogenomics in drug discovery and the clinical setting. In 2007, Paules was senior author and Bushel was first author on a study published in *Proceedings of the National Academy of Sciences USA*, "Blood gene expression signatures predict exposure levels," conducted by a team of researchers from NIEHS and the University of North Carolina, Chapel Hill (UNC). (*Proc Natl Acad Sci USA* **104**: 18211–18216, 2007; doi: 10.1073/pnas.0706987104)

In those experiments, the researchers found that the signature gene lists were able to predict exposure to toxic versus nontoxic doses of acetaminophen with very high accuracy (88.9–95.8 percent); the more traditional predictors, of clinical chemistry, hematology, and pathology were only 65 to 80 percent accurate.

As part of the study, the NIEHS researchers compared the animal data with data from RNA in blood drawn from individuals who had been admitted to the UNC emergency room for acetaminophen overdose intoxication. When they compared the toxic blood samples with the samples from normal healthy volunteers, the researchers saw a striking difference.

These results support the hypothesis that gene-expression data from peripheral blood cells can provide valuable information about exposure levels well before liver damage is detected by classical parameters. It also supports the potential use of genomic markers in the blood as surrogates for clinical markers of potential acute liver damage.

The NIEHS report on using genomic indicators to predict DILI is one of 11 papers published by MAQC-II consortium researchers in the same special issue of *The Pharmacogenomics Journal* (*Pharmacogenomics J* **10**:245–374, 2010). Although the NIEHS-led team was successful in its use of microarrays to identify predictors for clinical endpoints, some of the other teams experienced difficulty. An editorial in the journal observed, "Quality control and appropriate bioinformatics processing will remain a challenge for any new high-throughput molecular technology."

As phase II of the MAQC project nears completion, FDA plans a third phase: MAQC-III, also called the Sequencing Quality Control (SEQC) project. The SEQC project will assess the technical performance of next-generation sequencing platforms by generating benchmark datasets with reference samples. The project will also evaluate the advantages and limitations of various bioinformatics strategies in RNA and DNA analyses.

Bushel, Paules, and several other NIH members (from NIEHS and the National Center for Biotechnology Information) were among many co-authors of the MAQC-II project summary report that was published this summer (*Nature Biotechnol* **28**: 827–841, 2010).

NIEHS will continue this partnership with the FDA and other agencies to extend the successes of MAQC-II and to look further into the quality and reproducibility of next-generation sequencing through SEQC.

"This is another example of how NIH and FDA are working together to advance translational and regulatory science," said NIEHS Director Linda Birnbaum. "I'm proud of the role NIEHS investigators played in this important undertaking." ●

NIEHS: The mission of NIEHS, located in Research Triangle Park, N.C., is to reduce the burden of human illness and disability by understanding how the environment influences the development and progression of human disease. NIEHS traces its roots to 1966, when the U.S. Surgeon General announced the establishment of the Division of Environmental Health Sciences within the NIH. In 1969, the division was elevated to full NIH institute status. Since then, the NIEHS has evolved to its present status as a world leader in environmental health sciences with an impressive record of scientific accomplishments (see: http://www.niehs.nih.gov/about/research/index.cfm). For more information on NIEHS's intramural research, go to http://www.niehs.nih.gov/research/atniehs/index.cfm.